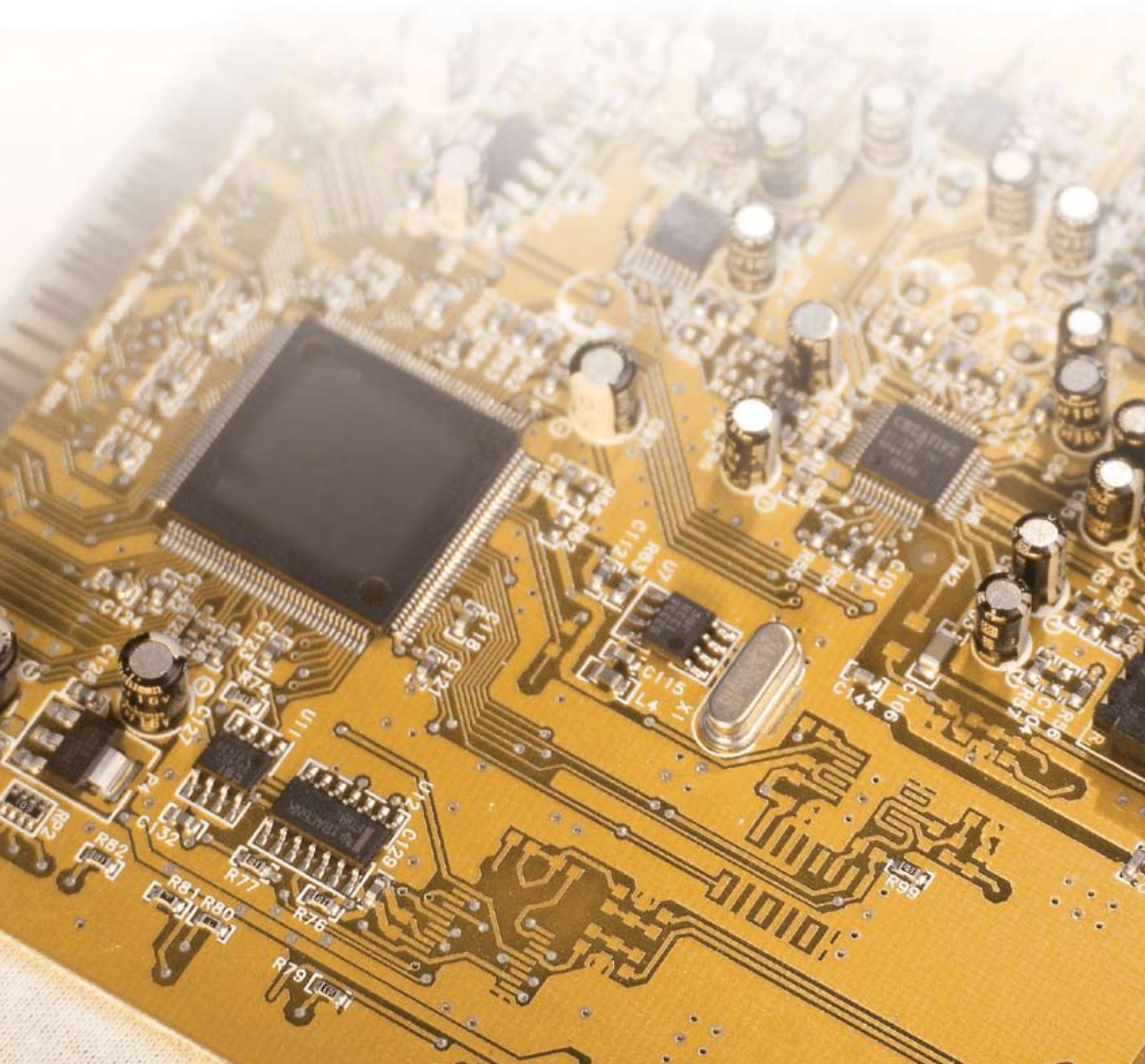
United States
Environmental Protection
Agency

TEST/QA PLAN FOR

Sabre Technical Services Chlorine Dioxide Gas Generator

Office of Research and Development
National Homeland Security
Research Center



EPA/Battelle Approval of

Test/QA Plan

For

EVALUATION OF SPORICIDAL DECONTAMINATION TECHNOLOGIES

February 2005

(SIGNATURES ON FILE)

Original signed by:

John C.S. Chang — 04/04/05
John C.S. Chang, Ph.D. — Date
Task Order Project Officer
U.S. EPA

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Eletha Brady-Roberts — 04/01/05
Eletha Brady-Roberts — Date
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Karen Riggs — Date
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Original signed by:

Zachary Willenberg — 03/30/05
Zachary Willenberg — Date
Quality Manager
Battelle

Test/QA Plan

for

EVALUATION OF SPORICIDAL DECONTAMINATION TECHNOLOGIES

February 2005

(SIGNATURES ON FILE)

Name David W. Skodack **Signature** David W. Skodack

Company Sabre Technical Services L.L.C.

Date 03/29/05

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ACRONYMS

ANOVA	two-way analysis of variance
ATCC	American Type Culture Collection
BSC	biosafety cabinet
BSL	biosafety level
C	Celsius
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CFU	colony forming unit
ClO_2	chlorine dioxide
cm	centimeter
d	deep
EPA	U.S. Environmental Protection Agency
FDA	Food and Drug Administration
h	high
ISO	International Organization for Standardization
L	liter
mL	milliliter
µL	microliter
MREF	Medical Research and Evaluation Facility
NHSRC	National Homeland Security Research Center
ppm	parts per million
QA	quality assurance
QC	quality control
QMP	Quality Management Plan
rpm	revolutions per minute
SD	standard deviation
SOP	standard operating procedure
TOPO	Task Order Project Officer
TSA	technical systems audit
TTEP	Technology Testing and Evaluation Program
UV	ultraviolet
w	wide

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A PROJECT MANAGMENT

A1 TECHNOLOGY EVALUATION ORGANIZATION

The technology evaluation will be performed by Battelle under the direction of the U.S. Environmental Protection Agency's (EPA) National Homeland Security Research Center (NHSRC) through the Technology Testing and Evaluation Program (TTEP). This test/quality assurance (QA) plan is based on a previously approved test/QA plan per the directive of the Task Order Project Officer (TOPO). The organization chart in Figure 1 shows the individuals from Battelle, the vendor, and EPA who will have responsibilities in the technology evaluation. The responsibilities of these organizations and individuals are summarized in the following subsections.

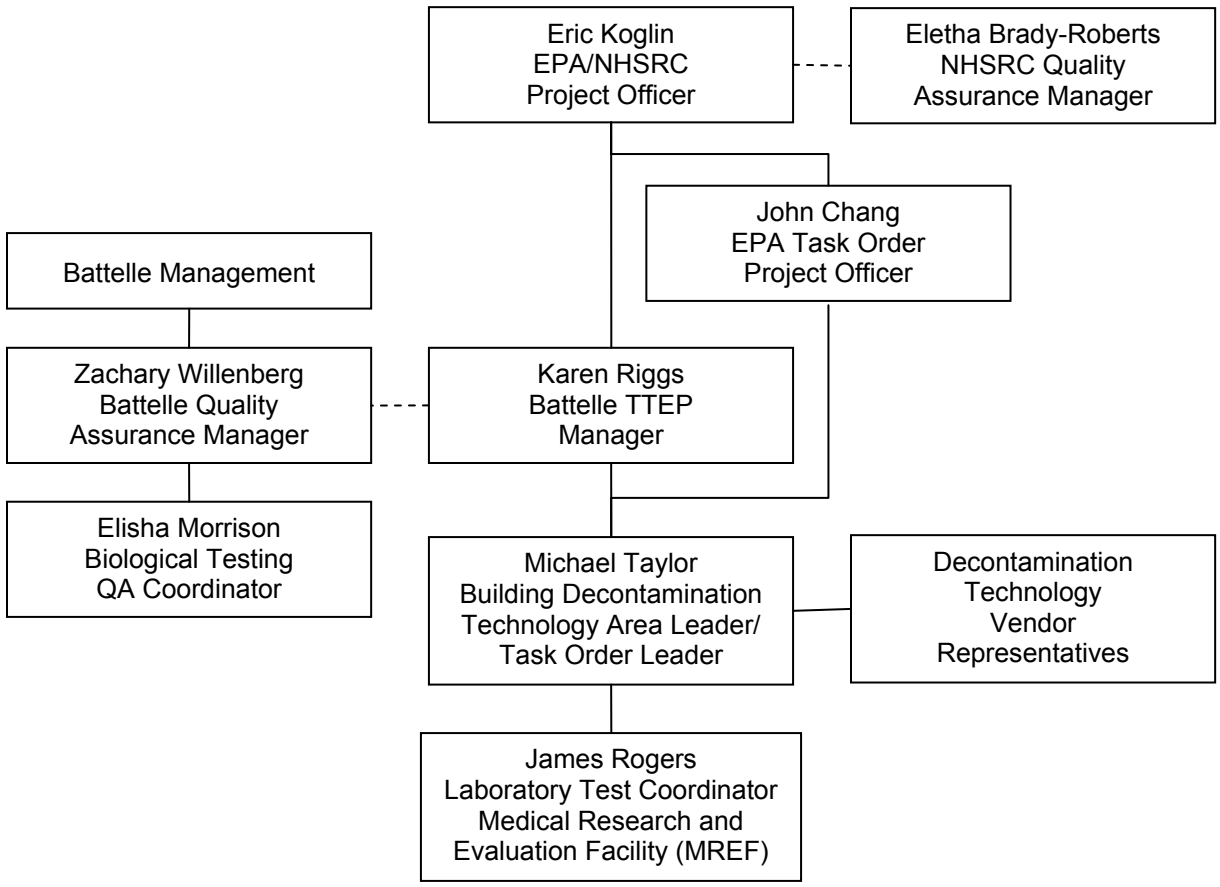


Figure 1. Organization Chart for the Sporicidal Decontamination Technology Evaluation

A1.1 Battelle

Dr. Michael Taylor is Battelle's Building Decontamination Technology Area Leader and Task Order Leader for this technology evaluation. He will have overall responsibility for ensuring that the technical, schedule, and cost goals established for testing and evaluation are met, and that the procedures employed for testing are consistent with TTEP guidelines. Dr. Taylor will serve as the primary interface for the TOPO. Dr. Taylor's responsibilities are to:

- Ensure that TTEP procedures are being followed.
- Select the appropriate laboratory or location for the evaluation.
- Prepare the draft test/QA plan and evaluation reports.
- Establish a test schedule.
- Revise this test/QA plan and evaluation reports in response to reviewers' comments.
- Keep the Battelle Program Manager informed of the progress and difficulties in planning and conducting the evaluation.
- Coordinate with the Battelle Quality Assurance Manager for the performance of technical and performance audits as required by Battelle or EPA Quality Management staff.
- Have overall responsibility for ensuring that this test/QA plan is followed.
- Respond to any issues raised in assessment reports and audits, including instituting corrective action as necessary.
- Establish a budget and schedule for the technology evaluation and direct the effort to ensure that budget and schedule are met.
- Coordinate distribution of final test/QA plan and evaluation reports.

Ms. Karen Riggs is Battelle's TTEP Manager. As such, Ms. Riggs will:

- Maintain communication with EPA's NHSRC Project Officer on all aspects of the program.
- Monitor adherence to budgets and schedules in this work.
- Provide the TOPO with monthly technical and financial progress reports.

- Review the draft test/QA plan.
- Review the draft evaluation reports.
- Ensure that necessary Battelle resources, including staff and facilities, are committed to the technology evaluation.
- Ensure that vendor confidentiality is maintained.
- Support Dr. Taylor in responding to any issues raised in assessment reports and audits.

Mr. Zachary Willenberg is Battelle's Quality Assurance Manager for TTEP. As such, Mr. Willenberg will:

- Review the draft test/QA plan.
- Maintain communication with EPA Quality Management staff for this program.
- Conduct a technical systems audit (TSA) at least once during the technology evaluation.
- Audit at least 10% of the evaluation data.
- Prepare and distribute an assessment report for each audit.
- Verify implementation of any necessary corrective action.
- Notify Battelle's TTEP Manager to issue a stop work order if internal audits indicate that data quality is being compromised. Notify the Task Order Leader if such an order is issued.
- Provide a summary of the QA/quality control (QC) activities and results for the evaluation reports.
- Review the draft evaluation reports.
- Ensure that all quality procedures specified in this test/QA plan and in the Quality Management Plan(1) (QMP) are followed.

Ms. Elisha Morrison will serve as Battelle's Biological Testing QA Coordinator and assist Mr. Willenberg as necessary.

Dr. James Rogers is Battelle's Laboratory Test Coordinator for this evaluation. His responsibilities are to:

- Coordinate with vendor representatives to facilitate the performance of the evaluation.
- Assist in preparation of the draft test/QA plan.
- Arrange for use of the test facility and establishment of evaluation schedules.
- Arrange for the availability of qualified staff to conduct the evaluation.
- Assure that the evaluation is conducted in accordance with this test/QA plan.
- Provide input into revision of this test/QA plan, evaluation report, and evaluation statement in response to reviewers' comments.
- Update the Battelle TTEP Manager and Task Order Leader on progress and difficulties in planning and conducting the evaluation.
- Coordinate with the Battelle Quality Assurance Manager for the performance of technical and performance audits as required by Battelle or EPA Quality Management staff.

A Medical Research and Evaluation Facility (MREF) Laboratory Facilities Coordinator will review and approve data and records related to facility operation. This Facilities Coordinator will:

- Review and approve all data and records related to facility operation.
- Provide input on facility procedures for the evaluation report.
- Provide requisite technical staff during the technology evaluation.
- Provide any safety training needed by Battelle, vendor, or EPA staff.

Battelle technical staff will support Dr. Taylor in planning and conducting the technology evaluation. These staff will:

- Ensure that the facility is fully functional prior to the times/dates needed in the technology evaluation.
- Adhere to the requirements of the test/QA plan and the program WMP in carrying out the technology evaluation.
- Support Dr. Taylor in responding to any issues raised in assessment reports and audits related to facility operation.

A1.2 Vendors

Vendors of the sporicidal decontamination technologies will:

- Provide input for preparation of the draft test/QA plan.
- Review this test/QA plan and approve the current version prior to the evaluation of their technology.
- Sign a Vendor Agreement specifying the respective responsibilities of the vendor and of Battelle in the evaluation.
- Provide information on the quantitative response of their sporicidal decontamination technology to aid in the planning of the evaluation.
- Provide the necessary equipment used for their sporicidal decontamination technology for use in the technology evaluation.
- Train Battelle and/or test facility staff in the operation of their sporicidal decontamination technology.
- Provide support, if needed, in use of their sporicidal decontamination technology during testing.
- Review their respective draft evaluation report.

A1.3 EPA

Mr. Eric Koglin is the EPA/NHSRC Project Officer for the EPA contract with Battelle, "Testing and Evaluation of Homeland Security-Related Technologies for the Measurement,

Sampling, Removal, and Decontamination of Chemical and Biological Agents" under which TTEP has been established.

Dr. John Chang is the EPA TOPO for Task Order 1113. As such, Dr. Chang will:

- Have overall responsibility for directing the evaluation process.
- Review the draft test/QA plan.
- Approve the final test/QA plan and any subsequent versions.
- Review the draft evaluation reports.
- Oversee the EPA review process on the draft test/QA plan and evaluation reports.
- Coordinate submission of evaluation reports for final EPA approval.

Ms. Eletha Brady-Roberts is the NHSRC Quality Assurance Manager for the TTEP. As such, Ms. Brady-Roberts will:

- Review the draft test/QA plan and any subsequent versions.
- Perform, at her option, one external TSA during the technology evaluation.
- Notify the EPA TOPO to issue a stop work order if an external audit indicates that data quality is being compromised.
- Prepare and distribute an assessment report summarizing the results of the external audit, if one is performed.
- Review the draft evaluation reports.

A1.4 Test Facility

The location for the technology evaluation described here will be Battelle's laboratories in Columbus and West Jefferson, Ohio. The Columbus facilities to be used are chemical laboratories equipped for safe handling of a wide variety of chemicals. The MREF, located in West Jefferson, has chemical and biological surety agent laboratories certified for use of chemical and biological warfare agents. Other test facilities could be used depending on the availability and capability of the facilities.

A2 PROBLEM DEFINITION/BACKGROUND

Among its responsibilities related to Homeland Security, the EPA has the goal of identifying methods and equipment that can be used for decontaminating indoor environments following a terrorist attack on a building using chemical or biological agents. In January 2003, EPA established the NHSRC to manage, coordinate, and support a wide variety of homeland security research and technical assistance efforts. The NHSRC is conducting tests to evaluate the performance of commercially-available products, methods, and equipment for decontamination of porous and non-porous indoor surfaces contaminated with biological or chemical agents.

The purpose of this testing is to generate objective performance data that can be used by building and facility managers, first responders, groups responsible for building decontamination, and other technology buyers and users to make informed purchase and application decisions. All potential users need unbiased, high-quality, objective third-party data and information in order to assess how well the available decontamination tools will meet their performance objectives while protecting human health and the environment. All testing and evaluation conducted through the TTEP is under the direction of EPA and is subject to the TTEP QMP. In performing each test, Battelle will follow the general procedures described in the QMP, and develop a separate test/QA plan that is specific for the type of decontamination technology being tested. This particular test/QA plan has been prepared for testing and evaluation of decontamination technologies that use gaseous or sporicidal decontamination agents [e.g. chlorine dioxide (ClO_2), hydrogen peroxide vapor, formaldehyde].

The objective of this test/QA plan is to describe laboratory test procedures that will be implemented to determine the efficacy of sporicidal technologies for removing or inactivating biological agents or surrogates on a range of representative indoor surfaces. This test/QA plan is specifically focused on decontamination of indoor surfaces typical of those found in a public building with the ultimate goal of providing technologies for restoring the building to a usable state following a terrorist attack. Decontamination of personnel or large equipment items (e.g. manufacturing equipment) is not covered in this test/QA plan. Decontamination technology testing and evaluation are being performed to generate data indicative of the technology

performance or efficacy. For the evaluation conducted under this test/QA plan, quantitative assessment of performance is accomplished by sampling and analysis of contaminants before and after the implementation of the decontamination technology. The performance parameters to be evaluated in the evaluation under this test/QA plan are discussed in the Appendix.

One or more biological agents (e.g., spores; vegetative cells, biotoxins) may be released inside a building during a terrorist attack. The highly persistent biological warfare agent, *Bacillus anthracis* Ames spores, was selected for this evaluation.

Indoor surfaces (e.g., carpet, laminate, wood) representing those found in a typical office building have been selected for use in evaluating the decontamination technology. The indoor surfaces selected include both porous and non-porous materials (see Section B1.3).

A3 TECHNOLOGY EVALUATION DESCRIPTION AND SCHEDULE

The overall objective of the evaluation called for under this test/QA plan is to determine the efficacy of the sporicidal decontamination technologies for removing/inactivating biological agents in or on typical indoor surfaces. Evaluation of each technology will be accompanied by careful monitoring of dwell time, decontamination agent concentration, temperature, relative humidity and other parameters that may impact decontamination efficacy.

A3.1 Applicability

This test/QA plan focuses on the evaluation of commercially available technologies for decontaminating indoor surfaces found in a typical office building or subway. This plan specifically focuses on building decontamination in the context of use by personnel responsible for decontamination after a terrorist attack. Toxic industrial chemicals, chemical warfare agents and/or biological warfare agents (including toxins) may pose a threat in the building contaminated by a terrorist attack. This plan focuses on the evaluation of technologies that are potentially applicable for decontaminating indoor surfaces contaminated with biological warfare agents (or toxins).

Technology evaluation testing requires a quantitative basis for establishing the performance of the tested technologies. For this evaluation, the performance of each of the decontamination technologies will be evaluated by comparing the amount of biological agent remaining on the indoor surface (carpet, wallboard, etc.) after decontamination with the amount of biological agent that was added to the indoor surface prior to decontamination.

A3.2 Scope

The overall objective of the technology evaluation described in this test/QA plan is to evaluate the performance of decontamination technologies using biological agents (including toxins) and surrogates under a range of realistic conditions. Testing may be conducted over ranges of temperature and relative humidity representing those that might be encountered in a decontamination situation in a building environment.

The performance parameters on which the decontamination technologies will be evaluated under this plan include:

- Log kill or efficacy [the logarithm (base 10) of the number of colony-forming units (CFUs) removed/destroyed by applying the decontamination technology].
- Surface damage caused by the decontamination technology.

The evaluation to be conducted under this plan is limited to detection of biological warfare agents (or surrogates) or toxins in or on individual samples (test coupons) of indoor materials. Testing will be conducted in two phases. The initial phase will take place in a naïve area (not a biological safety laboratory) and the second phase will take place in a biosafety level (BSL) 3 area.

In the initial phase, the decontamination technology will be coupled with the test chamber, associated monitoring devices will be installed in the test chamber and the vendor staff will train the MREF staff regarding use of the decontamination technology. In addition, the MREF staff will verify that all sub-systems comprising the testing apparatus are in working order.

The second phase of the testing entails evaluating the performance of the decontamination technology using live agent. This evaluation will be performed in a BSL-3 in the MREF.

A3.3 Schedule

The evaluation described in this test/QA plan is expected to commence within two weeks after this test/QA plan has been approved. It is anticipated that four weeks will be required to complete all testing for a single sporicidal technology. This schedule is predicated upon the vendor shipping the decontamination technology to Battelle and training Battelle personnel in the use of the equipment in accordance with the overall testing schedule.

A4 QUALITY OBJECTIVES

The performance parameters to be evaluated under this test/QA plan include:

- **Quantitative assessment of decontamination efficacy of sporicidal technologies.** CFUs in extracts of control test coupons are determined using standard plating techniques. Plate counts for three controls (inoculated with 10^8 spores per coupon but not decontaminated) is determined and each of these values is used to calculate the value N/N′ where N is the number of CFUs found on the control coupon and N′ is the number of CFUs found on the decontaminated coupon. The log [10] of each of these is calculated and the three logs are averaged to obtain a mean log kill ± standard deviation (SD). The log kill value is also efficacy discussed in Section B1.1. The log kill or efficacy value is used as an indication of the overall effectiveness of the decontamination technology. The closer the efficacy value is to 10^8 (the number of spores applied to the test coupon) the more effective or efficacious the treatment.
- **Qualitative assessment of residual biological agent and surrogate spores on test surfaces following decontamination and extraction.** Following the extraction to determine the efficacy value quantitatively, test coupons will be immersed in liquid growth medium contained in individual vials, cultured at the respective temperatures,

and bacterial growth in the vials will be observed visually at one and seven days after immersion. If the growth medium appears cloudy, then the conclusion is drawn that viable spores remained on the decontaminated samples following extraction. Growth could arise from spores added to the test coupon or reflect growth of indigenous organisms. Spores or other organisms giving rise to growth in the nutrient medium will be identified by standard culture techniques. A small sample of each positive culture will be streak plated onto tryptic soy agar plates and cultured overnight. The streak plates will provide information as to whether the organisms present are the same as those inoculated on the coupons, or other distinct microorganisms

- **Qualitative assessment of spore strips and biological indicators.** Following implementation of the decontamination technology the spore strips and indicators are placed in growth medium. The growth medium is examined at one and seven days post-decontamination for evidence of viable spores (medium becomes cloudy). No cloudiness indicates no viable spores which indicates 100% kill or a log kill of 10^6. The biological indicators and spore strips have been used in large-scale decontaminations to assess the completeness of decontamination. However, the spores on the biological indicators and spore strips are not *B. anthracis* spores but surrogates. In addition, the spore strips and biological indicators are more amenable to decontamination (biological indicators are a metal disc, spore strips are filter paper). The results for biological indicators and spore strips provide a means of comparing the effectiveness of the decontamination technology with other results obtained using the same or other decontamination technologies.
- **Changes in appearance of representative indoor surfaces.** Each test coupon is visually examined before and after application of the decontamination technology to assess whether or not the test coupon material exhibits physical changes (loss or change of color etc.). This information provides a general indication of how "caustic" the decontamination technology is and whether or not the contents of, for example, a subway station would be damaged or destroyed during decontamination.

Quantitative determinations in this study do not involve the use of analytical measurement devices. Rather, bacterial colonies will be enumerated manually and recorded. All other determinations will be qualitative. Specific information regarding the individual decontamination technologies is included in the Appendix.

A5 SPECIAL TRAINING/CERTIFICATION

These tests are expected to be conducted at the Battelle facility in West Jefferson, Ohio. That facility is described below. Alternative facilities could also be used, provided those facilities meet all the requirements for safety, security, and testing capability established by this test/QA plan.

A5.1 General Site Description

Evaluation of sporicidal decontamination technologies will be conducted at Battelle's MREF located in West Jefferson, Ohio, near Battelle's headquarters in Columbus, Ohio. The following section describes the MREF biofacility. The evaluation will be performed in accordance with Battelle's facility-specific methods and standard operating procedures (SOPs) that are cited where appropriate throughout this test/QA plan.

The MREF specializes in research, development, testing, and evaluation of medical countermeasures against highly pathogenic biological and highly toxic chemical materials. This facility is one of a very limited number of U.S. laboratories capable of studying aerosolized etiological agents in animal models under BSL-3 containment. This facility maintains state-of-the-art equipment, and professional and technical staffing expertise to safely conduct testing and evaluation of hazardous biological materials under the Food and Drug Administration's (FDA) Good Laboratory Practices Guidelines (21 CFR Part 58). The MREF operates in compliance with all applicable Federal, state, and local laws and regulations, including U.S. Army regulations and is routinely inspected by personnel from the appropriate government agency. Battelle operates the MREF in compliance with requirements contained in 32 CFR 626 and 627, Biological Defense Research Programs. The MREF facilities are ISO 9001 certified, accredited

by the American Association for the Accreditation of Laboratory Animal Care, and inspected and compliant with the U.S. Department of Agriculture, FDA, Drug Enforcement Agency, Ohio EPA, U.S. Army Safety Team, U.S. Army Inspector General, U.S. Army Medical Research Institute of Chemical Defense Safety and Chemical Operations Branch, U.S. Army Medical Research and Materiel Command Office of Animal Care and Use Review, Madison County Health Department, and Battelle's Institutional Animal Care and Use Committee. The MREF fully complies with all applicable U.S. Army Regulations, and Federal Government and State of Ohio regulations to conduct and support research, development, testing and evaluation studies using highly toxic chemical and pathogenic biological materials. The MREF is licensed to ship, receive, and handle select agents, as defined by the Centers for Disease Control and Prevention (CDC).

Testing outlined in this test/QA plan will be performed in the MREF BSL-3 facility, which was completed in 1995 and expanded to 31,000 square feet in 2002. The containment area within the facility is designed to meet or exceed the BSL-3 facility guidelines published by the CDC and National Institute of Health entitled *Biosafety in Microbiological and Biomedical Laboratories* (4th edition, 1999). Included are seven BSL-3 microbiology laboratories that contain multiple Class III biosafety cabinets (BSCs) and two autoclaves. Additional laboratories within this area include multiple microbiology laboratories equipped with Class II BSCs. Test procedures at the MREF are governed by established SOPs that are specified by facility, number, and title.

A5.2 Site Operations

Battelle operates the MREF in compliance with all applicable Federal, state, and local laws and regulations, including U.S. Army Regulations and the CDC. Battelle's facilities are certified through inspection by personnel from the appropriate government agency. Battelle's MREF is certified to work with both live and surrogate agents including bacterial endospores (e.g. *B. anthracis*), vegetative bacteria, and viruses. Additionally, the MREF is ISO 9001 certified, performs work under this ISO standard, and is monitored by regular outside ISO quality inspections.

A5.3 Training

Because of the hazardous materials involved in this technology evaluation, documentation of proper training and certification of the test personnel is mandatory before testing takes place. The Battelle Quality Assurance Manager, or a designee, must assure that documentation of such training is in place for all evaluation personnel before allowing evaluation to proceed.

All participants in this evaluation (i.e., Battelle, EPA, and vendor staff) will adhere to the security, health, and safety requirements of the Battelle facility in which testing will be performed. Vendor staff will train Battelle evaluation staff in the use of their decontamination technology, but will not be the technology users during the evaluation. To the extent allowed by the test facility, vendor staff may observe, but may not conduct, any of the technology evaluation activities identified in this test/QA plan.

Access to restricted areas of the test facility will be limited to staff who have met all the necessary training and security requirements. The existing access restrictions of the test facility will be followed, i.e., no departure from SOPs will be needed for this evaluation. All visiting staff at the test facility will be given a site-specific safety briefing prior to the start of any technology evaluation activities. This briefing will include a description of emergency operating procedures and the identification, location, and operation of safety equipment (e.g., fire alarms, fire extinguishers, eye washes, exits). Evaluation procedures must follow all safety practices of the test facility at all times. Any report of unsafe practices in this evaluation, by those involved in the evaluation or by other observers, shall be grounds for stopping the evaluation until the Quality Assurance Manager and testing personnel are satisfied that unsafe practices have been corrected.

A6 DOCUMENTATION AND RECORDS

Documentation of training related to technology testing, field testing, data analysis, and reporting is maintained for all Battelle technical staff in training files at their respective locations. The Battelle Quality Assurance Manager may verify the presence of appropriate

training records prior to the start of testing. Battelle will document training by the vendor with a form signed by the vendor. Battelle technical staff will have a minimum of a bachelor's degree in science/engineering or have equivalent work experience.

B MEASUREMENT AND DATA ACQUISITION

B1 EXPERIMENTAL DESIGN

B1.1 General Test Design

This test/QA plan specifies procedures for bench-scale testing to evaluate the performance of sporicidal decontamination technologies under specified operating conditions and ambient conditions for decontaminating small pieces (i.e. test coupons) of indoor materials to which biological agents and surrogates have been added.

Evaluation of the efficacy of a particular sporicidal technology for decontaminating a particular material will be accomplished by determining the differential mean reduction of viable agent or surrogate under an experimental treatment compared to mean reduction in the absence of the treatment (control). Treatments will be defined in terms of viable agent or surrogate, decontamination technology (or lack thereof) and operational use of the technology (e.g., concentrations, dwell times, flow/vent rates), and ambient conditions (e.g., temperature, humidity). Decontamination efficacy will be assessed by comparing the number of viable organisms remaining after decontamination with the number initially applied to the test surface. Efficacy will be expressed as the log (base 10) of result of dividing the mean number of viable organisms found on the control by the number of viable organisms found on a treated sample. Triplicate samples (e.g., 3 carpet, 3 wood, etc.) are subjected to the decontamination technology and an efficacy value for each sample is calculated and these three values are used to calculate a mean and SD.

In advance of each efficacy evaluation, a null hypothesis (H_O) will be formulated for the mean differential performance of the experimental treatment and control data:

$$H_O : R_{Treatment} - R_{Control} > c$$

$$H_A : R_{Treatment} - R_{Control} \leq c$$

Where:

$R_{Treatment}$ is the mean removal for the treatment group.

$R_{Control}$ is the mean removal for the control group.

c is a constant that could be zero if desirable to identify any significant removal or some threshold value for demonstrating minimum efficacy (e.g., 99.9999% removal).

The experimental treatment and controls will be defined according to desired planned comparisons. For any particular material, the planned comparisons may include:

- Efficacy of a decontamination technology under selected environmental conditions for a biological agent compared to a surrogate(s).
- Efficacy of a particular decontamination technology under selected environmental conditions and a particular biological agent for each of several different configurations of the technology (e.g., different concentrations, dwell times) or between use and absence of the technology.

After obtaining the experimental treatment and control data, a statistical comparison will be conducted. If sufficient evidence is found (at 95% confidence) that the experimental treatment mean removal exceeds the control mean removal above the applicable threshold "*c*", the treatment will be concluded to be efficacious.

A parallel sensitivity analysis will be conducted to determine, based on the variability of the data collected, how large a true difference in efficacy would be highly likely (90%) to have been identified as statistically significant by this hypothesis test.

Throughout the evaluation, the test coupons will be randomly assigned to control and experimental groups. Each decontamination technology will be applied in a manner consistent with the manufacturer's recommendations. The technology vendor will provide the equipment and training regarding application of their technology.

The statistical approaches for these analyses are discussed in Section B6.2.

B1.2 Scale of Testing and Testing Apparatus

The parameters listed above will be evaluated during bench-scale testing in the laboratory. A decontamination test chamber, a Compact Glove Box 830-ABC (Plas Labs, Inc., Lansing, MI; see Figure 2) will be used for exposing the test coupons to the decontamination technology. This test chamber has dimensions of 71 cm w x 59 cm d x 74 cm h (28" x 23" x 29") and outer dimensions of 110 cm w x 61 cm d x 79 cm h (43" x 24" x 31"). The test chamber has a total volume of 317L (11.2 cubic feet). The test chamber also has a top opening of 43 cm x 58 cm (17" x 23") and an attached transfer chamber that is 30 cm (12") long and an

inner diameter of 28 cm (11”). Glove ports are available for working in the test chamber. The test chamber may need to be modified, in accordance with the vendor, to accommodate the decontamination technology. The decontaminant (fumigant, foam, liquid, gel) will be directed from the vendor’s system into the test chamber under the conditions (dwell time, decontaminant concentration, temperature, and humidity) specified by the vendor. The performance tests will use test coupons that are approximately 1.9 cm w x 7.5 cm long (¾” x 3”); multiple coupons of each indoor material will be contaminated with the agent/surrogate, placed into the test chamber and then treated with the decontamination technology. Blank (i.e., uncontaminated) and control (i.e., contaminated but not decontaminated) coupons will also be prepared for each test material, and results obtained for these coupons will be utilized along with the data resulting from the analyses of post-treatment samples to calculate decontamination efficacy. This evaluation methodology comprises a highly controlled, reproducible approach to assess decontamination efficacy, while simulating a realistic, small-scale application of the decontamination technology.

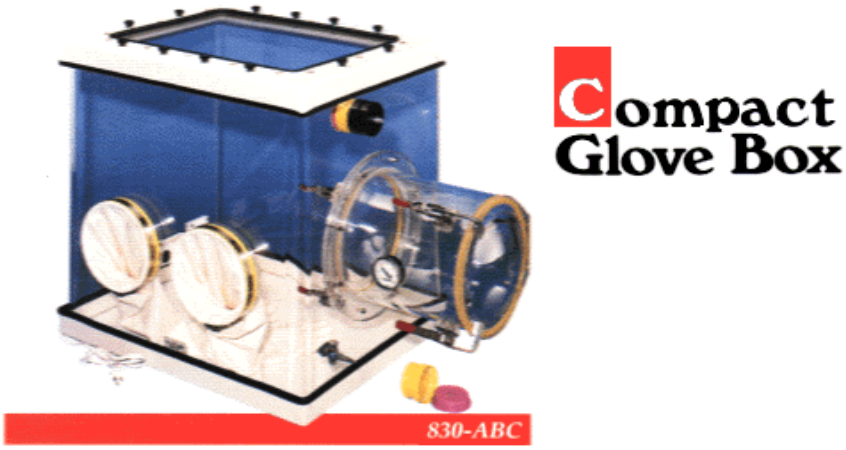


Figure 2. Compact Glove Box for the Sporicidal Decontamination Technology Evaluation

B1.3 Test Surfaces

A subset of the various structural, decorative, and functional surfaces that may be found inside an office building will be used for evaluating sporicidal decontamination technologies. The surface materials to be used include both non-porous and porous surfaces representing a variety of materials. Test coupons (typically measuring 1.9 cm x 7.5 cm) will be prepared from

larger pieces of stock material. The representativeness and uniformity of the test materials are critical attributes to assure reliable evaluation results. Representativeness means that the materials used are typical of such materials used in buildings in terms of quality, surface characteristics, structural integrity, etc. Uniformity means that all test pieces are essentially equivalent for evaluation purposes. Representativeness will be assured by selection of test materials that meet industry standards or specifications for indoor use, and by obtaining those materials from appropriate suppliers. Uniformity will be maintained by obtaining a large enough quantity of material that multiple test samples can be obtained with presumably uniform characteristics (e.g., test coupons will be cut from the interior rather than the edge of a large piece of material), or by using standardized coupons where available. The test surfaces that will be used are listed below with their corresponding sample identification codes in parentheses:

- Painted (latex, semi-gloss) concrete cinder block (PC)
- Painted (latex, flat) wallboard paper (PW)
- Decorative laminate (DL)
- Galvanized metal ductwork (GM)
- Glass (GS)
- Bare pine wood (BWD)
- Industrial carpet (IC)

B1.4 Biological Agents and Surrogates

The biological agent to be used in the evaluation under this test/QA plan has been selected based on an evaluation of potential threats to buildings.[3] The evaluation considered availability, lethality, potential delivery pathways and persistence of potential agents.

The biological agent used in evaluating the sporicidal decontamination technology will be *B. anthracis* Ames strain spores. The biological surrogates will be used to establish correlations between the decontamination efficacy of surrogates and agents. To provide correlations with the *B. anthracis* results, the surrogates *Bacillus subtilis* (ATCC 19659) and *Geobacillus stearothermophilus* (ATCC 12980) will be used. *B. anthracis* Ames strain spores and surrogate spores will be prepared and characterized according to MREF SOPs.

Both spores (*B. subtilis* and *G. stearothermophilus*) have commonly been used as surrogates for *B. anthracis* in decontamination technology testing. The *G. stearothermophilus* surrogate exhibits comparatively high resistance to various sporicidal decontaminants. The *B. subtilis* (ATCC 19659) surrogate is the most commonly used surrogate for *B. anthracis*.

Two types of biological indicators will be used under this test/QA plan. A commercial spore strip of the same spore type [*B. subtilis var niger* (*B. atrophaeus* ATCC 9372) on paper backing manufactured by Raven Biological Laboratories] as those used during decontamination of U.S. Postal Service facilities contaminated with *B. anthracis* will be used. In addition, biological indicators containing *B. stearothermophilus* and *B. subtilis* (Apex Laboratories, Inc.) will be included. Each of these biological indicators typically contains a spore population of approximately 10^6 spores on a stainless steel disc packaged in a Tyvek® envelope.

B1.5 Temperature and Relative Humidity Conditions

Each sporicidal decontamination technology may require different temperature and humidity conditions in the space to be decontaminated. The temperature and the relative humidity will be controlled and monitored during the decontamination process. Specific operating parameters and conditions for each sporicidal decontamination technology are specified in the Appendix.

B1.6 Surface Damage

The effect of the decontamination technology on the test coupons will be evaluated during the efficacy evaluation procedure. Before and after decontamination of the test coupons, the appearance of the decontaminated coupons will be visually inspected, and any obvious changes in the color, reflectivity, and apparent roughness of the coupon surfaces will be recorded in the evaluation. This comparison will be performed for each of the test materials, before extraction or sampling of the decontaminated test coupons.

B2 METHODS REQUIREMENTS AND PROCEDURES

B2.1 Agents

B. anthracis (Ames), *B. subtilis* (ATCC 19659) and *G. stearothermophilus* (ATCC 12980) spores will be prepared according to established MREF procedures. [4, 5] Working stock suspensions of each spore type will be prepared at a target concentration of approximately $1x10^9$ CFU/mL.

B2.2 Coupon-Scale Testing

B2.2.1 Preparation of Test Materials

Each of the test coupons will be cut to 1.9 cm x 7.5 cm size from the interior of a large piece of test material. Edges and damaged areas will be avoided in cutting test coupons. The test coupons will be visually inspected prior to inoculation with the biological agent or surrogates and any surface anomalies will be recorded. Specifications of each test coupon will be recorded. On each evaluation day, each coupon will be assigned a unique identifier code by the evaluation staff for traceability. Prior to the application of the biological agent or surrogate, the surface of each test coupon will be wiped with 70% isopropanol or ethanol to kill endogenous microorganisms. This is intended to minimize contamination by microorganisms other than those being evaluated. To ensure further cleanliness and prevent contamination of test surfaces, sterile technique will be exercised during all phases of handling the test coupons.

B2.2.2 Application of Biological Agents to Test Coupons

Application of biological agent/surrogates to test coupons will be performed in a BSC III according to established MREF procedures. [6] Test coupons will be placed lying flat in the cabinet and contaminated at challenge levels of approximately 1×10^8 CFU per coupon. A 100 µL aliquot of a stock suspension (approximately 1×10^9 CFU/mL) of spores will be dispensed using a micropipette as small droplets across the surface of the test coupon. After contamination with biological agent or surrogate suspension, the test coupons will remain overnight and undisturbed in the BSC III to dry.

B2.2.3 Confirmation of Surface Application Density

To confirm the application density of biological agents and surrogates, the respective spore suspensions used to contaminate the coupons will be re-enumerated on each day of use. This enumeration will be carried out as described in Section B2.2.5.

B2.2.4 Application of the Decontamination Technology, Monitoring of Test Procedures

One day following application of biological agent or surrogate, inoculated test coupons intended for decontamination (including one blank) will be transferred into the test chamber that has been coupled with the decontamination technology. The inoculated control coupons (not decontaminated) and one blank will be left undisturbed in the BSC. The decontamination technology will be applied in accordance with the vendor's instructions. The concentration of the gaseous decontaminant, dwell time, relative humidity, and temperature will be controlled and monitored as described in the Appendix. Following decontamination, the test chamber will be cleared using the vendor-supplied method for neutralization of decontamination chemicals.

B2.2.5 Determination of Decontamination Efficacy

The efficacy of a sporicidal technology for neutralizing/inactivating biological agents on indoor surface materials will be determined. For building decontamination, the amount of biological agent that remains following decontamination needs to be ascertained since residual agent could present a potential health risk for building occupants. The performance or efficacy of the sporicidal decontamination technology will be assessed by comparing the number of viable organisms remaining after decontamination with the number actually added to test coupons. For biological agents, there is currently no determined safe level for remaining residual organisms; [(2)] however, a traditional approach for qualifying decontamination performance is the assessment of growth of biological indicators pre-positioned inside the space being fumigated. Typically, biological indicators contain spore loads of approximately 1×10^6; therefore, complete neutralization of these biological indicators results in a 6-log kill. As stated above there is no officially recognized clean level for building decontamination; however, biological indicators

(used in other decontamination efforts) will also be used for this evaluation as a means for evaluating the efficacy of the sporicidal decontamination technology.

The decontaminated, control, and blank coupons will be placed individually in a sterile 50 mL conical vial to which 10 mL of sterile phosphate-buffered saline containing 0.1% Triton X-100 has been added. The purpose of the Triton X-100 is to minimize clumping of spores. For spore extraction, the tubes will be agitated on an orbital shaker for 15 minutes at approximately 200 rpm at room temperature. Each tube will then be heat-shocked for 1 hour at 60º C to 65º C (for *B. anthracis* and *B. subtilis*) or 90º C to 95º C (for *G. stearothermophilus*) to kill vegetative bacteria. Following the heat-shock, 1.0 mL of the extract will be removed and a series of dilutions through 10^{-7} will be prepared in sterile water. An aliquot (100 µL) of the undiluted extract and each serial dilution will be plated onto tryptic soy agar plates in triplicate, allowed to dry, and incubated overnight at 35º C to 37º C for *B. anthracis* and *B. subtilis* and at 55º C to 60º C for *G. stearothermophilus*. Plates will be enumerated within 18 to 24 hours of plating as described in References 7 and 8. The number of CFU/mL will be determined by multiplying the average number of colonies per plate by the reciprocal of the dilution. Data will be expressed as mean $\pm$ SD of the numbers of CFUs observed. To calculate the percent recovery of spores following treatment, the calculated number of spores remaining on the decontaminated coupons will be divided by the calculated number of spores on the control coupons that were not subject to decontamination.

Decontamination efficacy will be calculated as the log reduction (mean ± SD) in viable organisms achieved by the decontamination technology. Efficacy (E) for biological agents or surrogates will be calculated as

$$E = \log (N/N')$$

Where N is the number of viable organisms recovered from the control coupons (i.e., those not subjected to decontamination), and N′ is the number of viable organisms recovered from the test coupons after decontamination.

A separate efficacy calculation will be made for each of the surface materials for the biological agent and surrogates. Percent recovery (mean ± SD) will be calculated for each type of test material inoculated with each biological agent/surrogate by dividing the number of

biological organisms in the treated sample by the number of biological organisms in the control (non-decontaminated). For each material and agent/surrogate combination, a mean and range of the efficacy values will be reported. Thus, the primary efficacy results from the coupon testing will be a matrix table in which each entry shows the mean and range of efficacy results for one of the agents/surrogates on one of the surface materials.

Based on previous decontamination studies, it is assumed that 100% recovery of spores from the inoculated test coupons will not be achieved; therefore, viable spores may remain on the test coupons. A qualitative assessment of these spores will be performed to determine whether viable spores remain on the decontaminated test coupons. Following the extraction process described above, each coupon will be transferred into a sterile 50 mL conical tube containing 20 mL of tryptic soy broth culture medium. These vials will be cultured at the appropriate temperature for *B. anthracis* or surrogates to encourage viable spore germination and subsequent proliferation of vegetative bacteria. At one and seven days post-decontamination the tubes will be visually assessed qualitatively for viability. A cloudy culture medium will indicate "growth" of viable spores, while clear culture medium will indicate "no growth." In the seven day samples where growth is observed, a loop of the culture medium will be cultured on tryptic soy agar plates using a standard streak plate technique. This culturing on tryptic soy agar plates will provide a means to determine whether the observed growth in the liquid culture medium is due to the *B. anthracis* or surrogate applied to the coupon, a contaminant, or both.

The biological indicators and spore strips will be cultured in tryptic soy broth and assessed qualitatively at one and seven days post-decontamination for growth or no growth. However, further culturing of any positive samples on tryptic soy agar plates will not be performed.

B2.2.6 Observation of Surface Damage

Following application of the decontamination technology, each test surface will be examined visually to establish whether use of the decontamination approach caused any obvious damage to the surface. Observation of surface damage will be performed immediately after completion of the decontamination process but before post-decontamination sampling to assess

efficacy. If wetted by the decontamination process, the test surface will be allowed to dry before any inspection for damage. Visual inspection of the surface will then take place through side-by-side comparison of the decontaminated test surface and the control coupons of the same test material. Differences in color, reflectivity, and roughness will be assessed qualitatively and observations will be made by the evaluation staff and recorded**.**

B3 QUALITY CONTROL REQUIREMENTS

Quantitative standards do not exist for biological agents and surrogates. The confirmation procedure, controls, blanks, and method validation efforts will be the basis of support for biological evaluation results.

B4 INSTRUMENT CALIBRATION AND FREQUENCY

The equipment needed for the evaluation will be maintained and operated according to the quality requirements and documentation of the evaluation facility. Relative humidity and temperature in the test chamber will be monitored using a NIST-traceable digital thermometer – hygrometer. The accuracy of the instrumentation used to monitor the decontamination reagent (e.g. fumigant) will be verified as indicated in the Appendix.

B5 DATA ACQUISITION REQUIREMENTS

There are no data needed for this project implementation that are obtained from non-measurement sources such as computer databases, programs, literature files or historical databases.

B6 DATA MANAGEMENT

Data acquisition during the evaluation includes proper recording of the procedures used in the evaluation to assure consistency in the evaluation and adherence to this test/QA plan; documentation of sampling/evaluation conditions; recording observations regarding the condition of the surface of each coupon before and after the decontamination process; and recording of efficacy results and evaluation conditions. Data acquisition will be carried out by the Battelle testing staff manually and recorded immediately in a consistent format throughout all evaluations. All written records will be in ink and any corrections to recorded data will be made with a single line through the original entry. The correction will then be entered, initialed, and dated by the person making the correction. Any non-obvious correction will include a reason for the correction. Strict confidentiality of evaluation data will be maintained.

B6.1 Efficacy Calculations

For biological agents and surrogates, decontamination efficacy will be calculated as (described in Section B2.2.5) the reduction in viable organisms achieved by the technology.

B6.2 Statistical Analysis

For each material and species combination, calculating log reduction values will result in a total of 63 efficacy values (that is three coupons for each of seven materials analyzed in triplicate). In cases where no viable colonies remain after decontamination, one colony will be assumed to be present for the purpose of this calculation. A two-way analysis of variance (ANOVA) model with main effects for each test organism and test material and interactions will be fitted to the efficacy data. This model will be used to compare each mean to zero, compare each surrogate to a selected organism, for example *B. anthracis* (for a specific material), and compare each surrogate to a selected organism for porous and non-porous materials. T-tests or statistical contrasts will be used for the comparisons, with no adjustment for multiple comparisons. The ANOVA model is fitted using the SAS® (Version 8.2) GLM procedure.

The evaluation results will be compiled in a report. The report will briefly describe TTEP and evaluation procedures as well as all evaluation data and observations. The preparation

of the draft report, review of the draft report, the revision of the draft report, final approval, and the distribution of the final report, will be conducted as stated in the QMP.

C ASSESSMENT/OVERSIGHT

C1 ASSESSMENTS AND RESPONSE ACTIONS

Battelle's Quality Assurance Manager will audit at least 10% of the evaluation data. The Quality Assurance Manager will trace the data from initial acquisition, through reduction and statistical comparisons, to final reporting. All data calculations will be checked.

C1.1 Technical Systems Audit

Battelle's Quality Assurance Manager or designee will perform one TSA during the evaluation. The TSA is to ensure the evaluation is performed in accordance with the TTEP QMP and the test/QA plan and that QA/QC procedures are implemented. The Quality Assurance Manager may review evaluation methods, compare test procedures to those specified in this test/QA plan, and review data acquisition and handling procedures. The Quality Assurance Manager will prepare a TSA report and the findings must be addressed either by modifications of test procedures or by documentation in the evaluation records and final report. At EPA's discretion, EPA QA staff may also conduct an independent on-site TSA during the evaluation. The EPA TSA findings will be communicated to evaluation staff at the time of the audit, and documented in a TSA report. These findings must be addressed as stated above.

C1.2 Performance Evaluation Audits

No performance evaluation audit will be performed for biological agents and surrogates, as quantitative standards for these biological materials do not exist. The confirmation procedure, controls, blanks, and method validation efforts will be the basis of support for biological evaluation results.

C2 REPORTS TO MANAGEMENT

Each assessment and audit will be documented in accordance with the QMP. Assessment reports will include the following:

- Identification of any adverse findings or potential problems.

- Space for response to adverse findings or potential problems.
- Possible recommendations for resolving problems.
- Citation of any noteworthy practices that may be of use to others.
- Confirmation that solutions have been implemented and are effective.

During the course of any assessment or audit, the Quality Assurance Manager will identify to the technical staff performing experimental activities any immediate corrective action that should be taken. If serious quality problems exist, the Quality Assurance Manager is authorized to stop work. Once the assessment report has been prepared, the Building Decontamination Technology Area Leader or Task Order Leader will ensure that a response is provided for each adverse finding or potential problem, and will implement any necessary follow-up corrective action. The Quality Assurance Manager will ensure that follow-up corrective action has been taken.

D DATA VALIDATION AND USABILITY

Records generated during the evaluation will receive a QC/technical review before these records are used to calculate, evaluate, or report results. This review will be performed by a Battelle technical staff member other than the person who originally generated the record. Evaluation staff will be consulted as needed to clarify any issues about the data records. The review will be documented by the person performing the review by adding his/her initials and date to a hard copy of the record being reviewed. This hard copy will then be returned to the Battelle staff member who generated or who will be storing the record.

E REFERENCES

1. Quality Management Plan (QMP) for the Technology Testing and Evaluation Program (TTEP), Version 1, prepared by Battelle, Columbus, Ohio, January 2005.

2. Raber, E., Jin, A., Noonan, K., McGuire, R., and Kirvel, R.D. Decontamination Issues for Chemical and Biological Warfare Agents: How Clean is Clean Enough? *Int. J. Environ. Health Res.,* 11, 128-148 (2001).

3. Decontamination Technology Testing and Evaluation: Task 1: Technology Identification and Selection, prepared for the U.S. EPA under Task Order 1113 by Battelle, Columbus, Ohio, December 17, 2004.

4. Battelle MREF SOP Number: MREF. X-074, "Standard Operating Procedure (SOP) for the Production of *Bacillus anthracis* Spores."

5. Battelle MREF SOP Number: MREF. X-093, "Standard Operating Procedure (SOP) for the Production of *Bacillus anthracis* Spores in a Small Fermentor."

6. Battelle MREF Facility Safety Plan Annex 12 to Appendix B, "Guidelines for the Use of Class II and Class III Biological Safety Cabinets in the MREF Biofacility."

7. Battelle MREF SOP Number: MREF. X-054, "Standard Operating Procedure (SOP) for Enumeration of BL-2 and BL-3 Bacterial Samples via the Spread Plate Technique."

8. Battelle MREF SOP Number: MREF. X-112, "Standard Operating Procedure (SOP) for Interpreting and Calculating Enumeration Data for Biological Decontamination Testing."

APPENDIX

DETAILED DESCRIPTIONS OF DECONTAMINATION TECHNOLOGIES AND OPERATING PARAMETERS FOR EFFICACY EVALUATION

Sabre Technical Services Chlorine Dioxide Fumigant Technology

General Description:

ClO_2 is not stable as a compressed gas and, therefore, vaporous ClO_2 must be produced on-site. Decontamination technologies that utilize vaporous ClO_2 typically include the equipment and chemicals for on-site generation, delivery, removal and neutralization of ClO_2. In addition, ClO_2 generation technologies may require concurrent use of equipment for establishing and maintaining specific temperature and humidity conditions that are required for safe and effective operation of the decontamination process.

Sabre Equipment Description and Operating Parameters

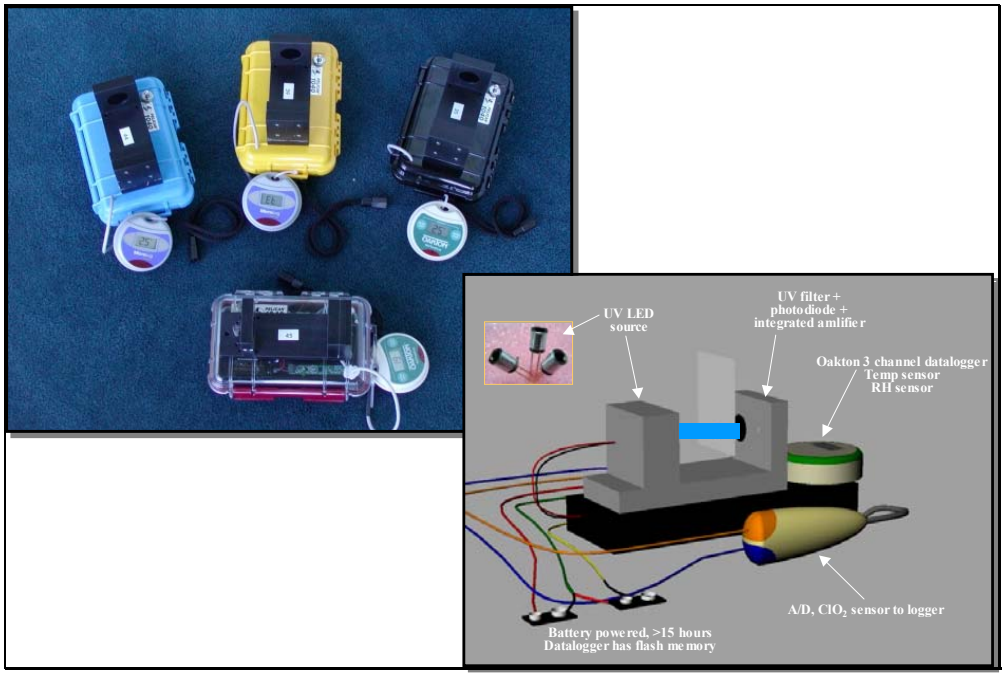
The Sabre equipment includes a 20.3 cm x 20.3 cm (8" x 8") base onto which is mounted a 15.2 cm x 15.2 cm (6" x 6"), 91.4 cm (36") high sparging column. A 19 L (5 gallon) container (vented through a sodium thiosulfate trap, container placed in an over pack for safety) containing 15 L of an aqueous solution consisting of 3g/L of ClO_2 plus 1000 ppm of chlorite is prepared on-site. The ClO_2 solution is pumped (using a peristaltic pump) into the sparging column and air from the test chamber is pumped into and through the column to sparge the ClO_2 from the liquid into the air stream; the air stream re-enters the glove box to establish the desired gaseous ClO_2 concentration. Liquid introduction from the reservoir of ClO_2/chlorite solution to the sparging column is initially at the rate of 60 mL per minute; when the desired ClO_2 concentration in the test chamber is achieved, the liquid introduction into the sparging column is decreased to 3 mL per minute. The spent liquid exiting the sparging column is collected in a reservoir. The air from the chamber is recirculated into and out of the sparging column. Temperature for the decontamination is held in the range of 23.9-35° C and the relative humidity should be held in

the range of 23.9-35° C. A nebulizer (supplied by Battelle for this test) is used to establish the desired humidity level in the test chamber. A constant temperature bath is employed to maintain the temperature of the liquid entering the sparging tower. The parameters of temperature and humidity will not be varied in this evaluation. Total treatment time is 3 hours for 3,000 ppm ClO_2 in order to achieve a CT of 9,000.

At the end of the decontamination test the ClO_2 in the system is removed by pumping through a carbon adsorption column.

Description of the Method for Real-Time Monitoring of ClO_2

The concentration of ClO_2 inside the glove box will be monitored using a portable ultraviolet (UV) spectrometer (developed by DARPA) for real-time monitoring of the concentration of ClO_2 in air. The UV spectrometer measures the strong 360 nm UV absorption of ClO_2 gas, generates a 4-20 mA signal and, based on the absorption data, the ClO_2 gas concentration is calculated. The UV optical beam is produced by a low power light emitting diode and is detected by a photodiode. A drawing of the monitor assembly is shown in the Figure below (lower panel), and a photograph of the as-built units is shown (upper picture).



The calibration of the UV spectrometer will be checked at the start of the study by comparing the concentrations indicated by the UV spectrometer (concentrations in the range of 1000 ppmv to 3000 ppmv) by sampling the air in the glove box and comparing the ClO_2 concentrations found using OSHA Method ID 202 [see www.osha.gov/dts/sltc/methods/inorganic/id202/id202.html (Note: This method was modified by reducing the volume of air captured to only 0.5 mL and increasing the potassium iodide capture solution volumes to 50 mL)] with concentrations determined using the DARPA UV spectrometer. The concentrations of ClO_2 as determined using the DARPA UV spectrometer will be deemed acceptable if the concentration determined using the DARPA UV spectrometer (over the ClO_2 concentrations of 1000 ppm to 3200 ppm) agrees within ± 30% of the corresponding concentration determined using OSHA Method ID 202. [Note: Data indicative of the performance of OSHA Method ID 202 will be included in the section of the report that describes the effort to calibrate the DARPA UV spectrometer.]